10/30/98

Clinical Pharmacokinetics Review of BLA 98-0286 (Enbrel, TNFR:Fc)

Pharmacokinetic Studies Summary:

1. Protocol 9125, Phase I/II Trial to Evaluate the Safety and Efficacy of Recombinant Human TNF Receptor (rhu TNFR:Fc) in Experimental Endotoxemia, an open, label parallel design study using 1, 5, 10, 15, 30, 60 mg/m2 single dose iv in healthy volunteers, N=13. A subgroup of patients (N=12) were given 10 or 60 mg/m2 and endotoxin to assess its effect on TNFR:Fc pharmacokinetics.

2. Protocol 9203, Phase II Multi-Center Trial to Evaluate the Safety and Efficacy of Recombinant Human TNF Receptor (rhu TNFR:Fc) in Sepsis Syndrome, a double blind, randomized placebo-controlled parallel designed study using 0.15, 0.45, or 1.5 mg/kg single iv dose in sepsis patients (N=85).

3. Protocol 16.0001, Phase I/II Trial to Evaluate the Safety and Efficacy of Recombinant Human TNF Receptor (rhu TNFR:Fc) in Human Immunodeficiency Virus-1 Infection, an open label parallel design study using 0.25, 1, or 2.5 mg/m2 single dose sc and then 0.125, 0.5 or 1.25 mg/m2 sc twice weekly for 8 weeks in HIV positive patients (N=7).

4. Protocol 16.0002, Phase I Study of Recombinant Human TNF Receptor:Fc (rhu TNFR:Fc) in Patients with Active Rheumatoid Arthritis.

5. Protocol 16.0006, Pharmacokinetic and Bioavailability Study of Radiolabeled Soluble TNF Receptor in Normal Volunteers in Active Crohn's Disease and in Active Rheumatoid Arthritis, using radiolabeled 1, 2, 4, 8, 10, 16, or 25 mg/m2 sc; 16 mg/m2 as a single dose iv; 10 or 25 mg as a single sc dose in an open label, parallel group design in healthy volunteers, RA or Crohn's disease patients (N = 15).

6. Protocol 16.0008, Multi-Center Study of Retreatment of Active Rheumatoid Arthritis with Recombinant Human TNF Receptor (rhu TNFR:Fc), an open label parallel design using 25 mg sc dose twice weekly in RA patients (N=28) DMARD-failing for 6 months.

7. Protocol 16.0010, A Pharmacokinetic and Absolute Bioavailability of Lyophilized TNFR:Fc in Normal Volunteers.

8. Protocol 16.0017, A Bioequivalence Study Comparing TNF Receptor (TNFR:Fc) from Two Different Manufacturing Sites, an open label randomized, crossover study using 25 mg dose sc in healthy volunteers (N=26).

Study Review:

1. Protocol 9125, Phase I/II Trial to Evaluate the Safety and Efficacy of Recombinant Human TNF Receptor (rhu TNFR:Fc) in Experimental Endotoxemia, an open, label parallel design study using 1, 5, 10, 15, 30, 60 mg/m2 single dose iv in healthy volunteers, N=13. A subgroup

of patients (N=12) were given 10 or 60 mg/m2 and endotoxin to assess its effect on TNFR:Fc pharmacokinetics.

This study was divided into two parts: part A which evaluated the safety and pk of a single iv dose in normal individuals and part B which evaluated the safety, activity and pk of 10 or 60 mg/m2 given iv to normal prior to a 4 ng/kg challenge of endotoxin. TNFR:Fc or placebo was given in a total volume of 100 ml over 30 minutes. When given endotoxin was administered iv immediately after TNFR:Fc over 1 to 2 minutes.

Pharmacokinetic data were fit to a two compartment model. AUC was computed using a trapezoidal method. Total body Clearance was determined as Dose/AUC and t1/2 was computed using the terminal elimination constant taken from the two-compartment model, see tables below for values. This method of calculating the t1/2 was different than that used in the other studies. The method using the two compartment model to determine the final elmination t1/2 is expected to yield larger a estimation of t1/2 than other means of its computation.

TNFR:Fc delayed fever and attenuated leukopenia and leukocytosis but did not antagonize other clinical effects of endotoxin. Both rhu TNFR:Fc doses decreased TNF-α bioactivity and IL-8, IL-1a and G-CSF levels. Low dose of TNFR:Fc decreased IL-6 levels. Without, endotoxin challenge, the t1/2 was calculated to be 73 hours (range 58 to 85 hours) and AUC and Cmax were observed to be proportionate to dose. With challenge at the high dose, clearance was increased, AUC and Cmax decreased, t1/2 was unchanged.

Dose, mg/m2	Cmax, ug/ml	AUC, ug-hr/ml	t1/2, hr	Clt, ml/hr/m2
1	0.4	45	85	23
5	2.1	159	71	32
10	3.8	305	63	33
15	8.8	586	58	26
30	14.3	1006	77	30
60	42.4	2056	82	29

Table of pharmacokinetic values for normal volunteers given TNFR:Fc and no endotoxin. As group size is only 2 or 3, no standard deviations are shown.

Dose, mg/m2	Cmax, ug/ml		AUC, ug-hr/ml		t1/2, hr		Clt, ml/hr/m2	
	E+	E-	E+	E-	E+	E-	E+	E-
10	3.8	3.8	368	305	63	76	32	33

Dose, mg/m2	Cmax, ug/ml		AUC, ug-hr/ml		t1/2, hr		Clt, ml/hr/m2	
60	22	42	1325	2056	82	61	46	29

Table illustrating the effect of endotoxin on the pharmacokinetics of TNFR:Fc. E+ is with endotoxin challenge and E- is without. As the group size in the non-challenged group are too small for statistical analysis, no standard deviation is shown.

2. Protocol 9203, Phase II Multi-Center Trial to Evaluate the Safety and Efficacy of Recombinant Human TNF Receptor (rhu TNFR:Fc) in Sepsis Syndrome, a double blind, randomized placebo-controlled parallel designed study suing 0.15, 0.45, or 1.5 mg/kg single iv dose in sepsis patients (N=85). No pharmacokinetic data were submitted in the study reports. Although various pharmacokinetic endpoints like clearance are reported in the summary documents, the original, raw data and methods of calculating the pharmacokinetic endpoints are not reported.

3. Protocol 16.0001, Phase I/II Trial to Evaluate the Safety and Efficacy of Recombinant Human TNF Receptor (rhu TNFR:Fc) in Human Immunodeficiency Virus-1 Infection. This study is an open label parallel design study in HIV positive (N = 7) patients given a loading dose (0.25, 1, or 2.5 mg/m2) sc and then 0.125, 0.5, or 1.25 mg/m2 sc, respectively, twice weekly for 8 weeks. Study dates Oct 1993 thru Sept 1994. Lot FXH0002A and FXH0002G.

HIV infected patients were given an initial loading dose and subsequently a maintenance dose which was one half the loading dose as indicated above. Following the initial dose, serum was collected for pharmacokinetics at 0, 1, 4, 24 48 and 72 hours after injection. Additional trough serum levels were collected at weeks 2, 4, and 6 just prior to injection and at 24, 48, 72, 168 (day 7) and 336 (day 14) hours after the final dose of TNFR:Fc.

No analysis of the pharmacokinetic results were provided in the individual study report; however, the results of an analysis were cited in the summary of studies. Two subjects —— and —— did not have their doses recorded and presumably belong to the 0.250/0.125 mg/m2 group. With repeated dosing, the majority of individuals showed accumulation in their steady-state levels suggestive of a decreased clearance. To illustrate this event, serum samples from comparable collection periods from week 7 were divided by those from week 1 and presented in the table below. Serum levels were standardized by dividing the serum level by dose before a ratio of serum levels by time was computed.

	—	—	—	—	—	—	—	—	—
24 h	5.8	2.2	11.6	1.3	ns	ns	6.5	1.7	2.0
48 h	3.3	1.7	6.6	1.4	ns	ns	5.7	2.9	1.4
72 h	2.5	1	4.9	1.9	ns	ns	3.8	1.4	2.3

Table of pharmacokinetic ratio of serum levels from week 1 and 7 for HIV infected individuals. (ns indicates no sample was available for analysis)

4. Protocol 16.0002, Phase I Dose-Escalation Study of Recombinant Human TNF Receptor (rhu TNFR:Fc) in Patients with Rheumatoid Arthritis. Lots FXH0002A, FXH0002C, FXH0002D, FXH0002H. Study dates May 1993 thru May 1994

This study was a single-center double-blind randomized placebo-controlled dose escalation study in RA patients using 4 , 8, 16, or 32 mg/m2 as a single iv dose and then 2, 4, 8, or 16 mg/m2 sc twice weekly for 4 weeks (N=15). The initial iv dose served as a loading dose which was followed by a sc dosing at one-half the iv dose (dosing groups are noted as 4/2, 8/4, 16/8 and 32/16 mg/m2). The iv dose was administered on day 1 of the study with the sc dosing begun on day 4. Initially, 4 patients were enrolled a dosing cohort with 1 or 4 to receive a placebo. The blind was broken after each cohort, and the patient given the placebo was then eligible to receive TNFR:Fc in an open-label manner. Additionally, patients were enrolled in the 16/8 and 32/16 mg/m2 as open-label dosing groups to obtain safety data.

Serum samples were analyzed from 15 patients. Limited serum samples were determined after iv and sc administration. Samples were taken just prior to treatment, immediately after the end of iv infusion and 1, 24, 48 and 72 hours post iv infusion. During sc treatment on days 8, 15, 22, and 29, serum samples were taken as trough levels, just prior to dosing and daily for 6 days (study days 30 - 35) after the last day of sc treatment. Monomeric receptor (TNFR) was detectable pre-injection in 12 patients. The iv data is characterized by some deficiencies: two patients have infeasible levels and an immediate post iv dosing sample is missing in another patient. An insufficient number of sample collection times were collected during the iv phase of the study to make the t1/2 estimates reliable. For sc dosing, serum levels are expected to rise during repeated sc dosing but were actually found to decline. Accordingly to the mathematical prinicples which form the basis for pharmacokinetics, dosing on the t1/2 should yield a steady-state serum level twice the initial level at 3 to 5 half-lives. Given the apparent t1/2 of 70 to 80 hours from the population pharmacokinetic analysis and other pharmacokinetic studies, patients should show successive rises in serum levels of TNFR:Fc upon repeated sc dosing twice weekly (ie., a dosing interval of 84 hours), but this was not observed. The reason for the variance of serum levels from that predicted by the t1/2 and dosing interval to that actually observed is not known. This decreasing level is the likely explanation for the reported increase in clearance reported by the sponsor in this study (see table below). The reported increase in clearance stand in sharp constrast to the decrease in clearance reported to occur in rhematoid arthritis patients by the population pharmacokinetic analysis. According to the population pharmacokinetic analysis, "patinets with RA had somewhat reduced clearance compared with subjects without RA. Assuming an age of 40 years, TNFR:Fc clearance would be 0.106 L/h in a subject without RA, compared with 0.0664 L/h in a patient with RA." After the 4 dosing interval, systemic levels appeared stable in a few cases but generally declined in a manner consistent with the t1/2 in the post sc dosing period. Significant levels of TNFR:Fc continued beyond the final observation during the post sc dosing (6 days) period. Generally, patients showed wide variation in their

serum levels.

Dose, mg/m2	N	Cmax. ug/ml	AUC. ug-hr/ml	Clt/F. L/hr/m2
2	3	0.2	17	0.361
4	3	0.5	38	0.280
8	6	0.6	43	0.645
16	3	1.8	158	0.351

Table of Average Values for Serum Levels of TNFR:Fc Given sc Twice Weekly after an iv Loading Dose to Pateints with Active Rheumatoid Arthritis.

5. Protocol 16.0006, Pharmacokinetic and Bioavailability Study of Radiolabeled Soluble TNF Receptor in Normal Volunteers, in Active Crohn's Disease and in Active Rheumatoid Arthritis, using radiolabeled 1, 2, 4, 8, 10, 16, or 25 mg/m2 sc; 16 mg/m2 as a single dose iv; 10 or 25 mg as a single sc dose in an open label, parallel group design in healthy volunteers, RA or Crohn's disease patients (N = 15). Lot FXH0003. Study dates Feb 1996 to Nov 1996.

This is an open, label Phase 1 study of the pharmacokinetics of TNFR:Fc in normal volunteers (N = 8), Crohn's disease patient (N = 1) and patients with active RA (N = 6, 3/dose). Single injection sc or iv were given using a variety of doses. Drug levels were assessed by tracer radiolabelling techniques or ELISA. Part A investigated the pharmacokinetics of a single dose given sc at 1, 2, 4, 8, or 16 mg/m2 with I^{125} TNFR:Fc to healthy volunteers; part B was a single dose given iv at 16 mg/m2 using I^{131} TNFR:Fc to active Crohn's disease patients; part C was a single sc dose of 10 mg (6 mg/m2) or 25 mg (16 mg/m2) in active RA patients.

Pharmacokinetic endpoints were determined using noncompartmental methods; AUC by the trapezoidal rule and t1/2 by last concentration/β. AUCT is the AUC to the last measured serum concentration and AUC is reported as extrapolated to infinity. Clt was determined by Dose/AUC. Serum samples were collected at pre-dosing (within 30 minutes), immediately ? al injection volumes (1.25 ml/each) and administered as 2 different sites. The single iv dose in part B (16 mg/m2) was infused over a 30 minute period.

Bioavailability could not be determined as no individual received both sc and iv doses. Peak drug levels after sc injection often occurred between days 3 and 4. The t1/2 could not be reliably determined as the duration of sampling (192 hours) was inadequate to characterize the terminal elimination phase. The t1/2 was reported by the sponsor to range from 84 to 171 hours. TNFR:Fc appeared to distributed to all organs incl: ding bone, liver, lungs, spleen and kidney. Doses of 1, 2, 4 and 8 mg/m2 appeared to exhibit a dose dependent reduction in Clt which reach a nadir at 8 mg/m2; no difference was apparent between 8 and 16 mg/m2. Due to the small numbers of subjects involved in the estimate, no definitive conclusion regarding the relationship

between Clt and dose can be determined at this time. Changes in the relative extent of absorption from the site of injection could also contribute to a decreased apparent clearance.

Pharmacokinetics results are presented below. Tmax was observed to be 48 (4/5 doses) or 60 hours in part A, 56 and 72 hours in part C.

Pharmacokinetic endpoint (AUC standardized by dose)	1 mg/m2 N =2	2 mg/m2 N=2	4 mg/kg N=2	8 mg/kg N = 1	16 mg/m2 N=1
AUC, ug-hr/ml (AUC/Dose)	11 (11)	50 (25)	119 (30)	182 (23)	304 (19)
Cmax, ug/ml	0.05	0.28	0.51	0.94	1.89
Clt, ml/hr/m2	234	135	104	88	100

Table of pharmacokinetic results from study 16.0006. Mean of sc dosing in healthy volunteers (part A)

Pharmacokinetic endpoints (AUC standardized by dose)	Part B, 16 mg/m2 iv N=1	Part C, 10 mg sc, N=3	Part C, 25 mg sc, N=3
AUC, ug-hr/ml (AUC/Dose)	267 (17)	80 (8)	296 (12)
Clt, ml/hr/m2	100	69	45
Half-life, hr	41	102	171
Cmax, ug/ml	6.3	0.4	1.2

Table of mean pharmacokinetic endpoints from parts B and C, study 16.0006.

6. Protocol 16.0008, Multi-Center Study of Retreatment of Active Rheumatoid Arthritis with Recombinant Human TNF Receptor (rhu TNFR:Fc), an open label parallel design using 25 mg sc dose twice weekly in RA patients (N=28) DMARD-failing for 6 months. Single serum samples were reported for this study of which 1 sample was collected during week 2, 2 samples during week 12 and 25 samples during week 24 of sc dosing. Sampling times after dosing are at listed in the study report, although a graph was provided by the sponsor indicating sampling serum levels and days after last dose. Assuming steady-state levels an apparent clearance of 57 ml/hr/m2 was reported.

7. Protocol 16.0010, A Pharmacokinetic and Absolute Bioavailability Study of Lyphilized Recombinant Human TNF Receptor (rhu TNFR:Fc) in Normal Volunteers. Lots FXH 001188.

This study was an open label crossover study using 10 mg dose sc and 10 mg iv in healthy volunteers (N=6; two groups of 3 each). Ten mg is approximately a dose of 5.7 mg/m2 given to a 70 kg individual. The study was considered a crossover study by route of administration rather than by formulation as subjects were given a sc followed by an iv infusion over 30 minutes. Injectins were separated by 28 days.

Pharmacokinetic endpoints were determined using noncompartmental methods. AUC was determined using the trapezoidal rule; half-life as -log(2)/β. The regression coefficient (β) was estimated using times of 72 hour and later for iv dosing and 144 hours and later for sc dosing. Bioavailability was computed as the ratio of AUC's by route (sc/iv).

Sampling times for the sc route beginning on day 1 of the study were collected over vaious time from 0 to 480 hours after injection. Sample collection was different for the two groups; the nujber of samples was decreased in the second cohort of individuals. See page 18, section 6.0 for details.

Sampling times for the iv beginning on day 28 of the study were collected between 0 and 312 hours after injection. Sample collection was different for the two groups; the nujber of samples was decreased in the second cohort of individuals. See page 19, section 6.0 for details.

The pharmacokinetic results are summarized in the table below:

Pharmacokinetic endpoint	SC (N=6)	IV (N=6)
AUC, µg-hr/ml	82	139
Tmax, hr	66	0.8
Half-life, hr	92	72
Bioavailability (%)	58	-

Table summarizing the pharmacokinetics after sc and iv administration in normal volunteers.

8. Protocol 16.0017, A Bioequivalence Study Comparing TNF Receptor (TNFR:Fc) from Two Different Manufacturing Sites, an open label randomized, crossover study using 25 mg dose sc in healthy volunteers (N=26).

Initial clinical material for TNFR:Fc were made at Immunex Corporation, ——— and later, in anticipation of commercial manufacturing needs, at ——— ———. The cross-over pharmacokinetic study was conducted to compare the kinetics and safety profiles of TNFR:Fc produced at each site using a 25 mg (16 mg/m2) single sc dose in healthy volunteers. Twenty-six healthy volunteers were randomly assigned to one of two treatment sequences based on the site of production: group 1 (— —day 1- ——— day 29) or

group 2 (——— day 1· ———day 29). Treatments were separated by 28 days. Blood samples were collected just prior to drug administration (time 0) and at the following times after sc injection: 2, 4, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168, 216, 264, 312, 384, 480. Whole blood was collected and serum samples used for the analytical assay (ELISA, limit of quantitation 0.3 ng/ml). The ELISA does not distinguish between TNFR:Fc and endogenous TNFR. Pharmacokinetics parameters were computed using noncompartmental techniques. A number of pharmacokinetic endpoints were determined: AUCT (AUC to last measured time point), AUC (AUC extrapolated to infinity), Tmax, Cmax (observed), t1/2 (as -log(2)/β), Clf (apparent total body clearance as Dose/AUC, aka Cl/F). The ANOVA to analyze the results included factors for sequence, subject within sequence, production site, day, as well as pharmacokinetic endpoints. Approximate 90% confidence intervals were calculated for the ratio of the site means. Equivalence was assessed by 90% confidence intervals using 0.8 and 1.25 as boundary conditions for log transformed data. Minor variations in sample collection time occurred in the course of the study; these do not significantly effect the results of the study. Only 4 females were enrolled in the study.

Types and grades of adverse events which occurred during the study were tabulated and summarized by body system and compared descriptively between the two manufacturing sites. A modified NCI Common Toxicity Criteria were used for grading adverse events. All subjects completed the study without any withdrawals. Two subjects required acetaminophen for headache, 1 subject was given cephalexin for a sore throat and the same subject was given diphenhydramine for hives. No differences were observed in adverse event rates between material produced at ——— or ———

The ratio of the AUCT mean for ——— relative to ——— material was 1.04 and the 90% confidence interval was 0.94 to 1.15. Similar results were demonstrated using AUC (mean 1.04 with confidence interval of 0.94 and 1.16) Therefore, the ——— and ——— material are pharmacokinetically equivalent as their values are inside the boundary of 0.8 and 1.25. Pharmacokinetic endpoints derived from the data are presented below:

Pharmacokinetic endpoints	——— site	——— site
AUCT, ug-hr/ml	231 ± 96.1	241 ± 76.0
AUC, ug-hr/ml	235 ± 96.9	245 ± 76.6
Tmax, hr	48 ± 19	49 ± 17
Cmax, ug/ml	1.64 ± 0.75	1.65 ± 0.66
t1/2, hr	72 ± 14	72 ± 14
Clt, ml/hr	131 ± 81	114 ± 42

Table of pharmacokinetic values for the cross-over pharmacokinetic equivalence study using

normal volunteers (mean ± SD).

Population Pharmacokinetics

For the population pharmacokinetic analysis, serum samples from 7 of 8 studies reported above were combined with data from protocols 16.0004, 16.0014 (given methotrexate) and 16.0016 (pediatric patients with active Juvenile Rheumatoid Arthritis). The data from study 16.0008 were not used in the population pharmacokinetic analysis. Altogether 10 studies were combined in ppk. Individual serum samples from various studies which were considered to be outliers were excluded from the population pk analysis. Using the ppk analysis, weight was found to influnce the estimate of the central volume of distribution. The central volume of administration was found to be 7.57 L and of steady-state volume of distribution 10.4 for a 70 kg individual. Clearance was reported to be 70 mg/h in patients with RA and 110 ml/h for healthy volunteers.

Martin D. Green

Martin D. Green, Ph.D.